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STUDY ON THE EXTRACTION PROCESS OF TOTAL ANTHRAQUINONES IN RADIX ET RHIZOMA RHEI AND THEIR ANTILIPEMIC EFFECTS

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Abstract

Background: Radix et Rhizoma Rhei has a gastric mucosal protective effect, major anti-gastritis and anti-peptic ulcer active constituents were emodin, aloe-emodin, chrysophanol, rhein, etc. The objective of the study was the extraction process of total anthraquinones in *Radix et Rhizoma rhei* and their antilipemic effects.

Materials and Methods: Orthogonal experiment, UV spectrophotometry and conventional antilipemic effect determination method were used to optimize the extraction process, and to determine the total anthraquinone content, as well as blood levels of total cholesterol, triglycerides, HDL and LDL.

Results: Ethanol concentration, extraction time and ethanol amount had significant influences on the extraction of total *Radix et Rhizoma rhei* anthraquinones, total *Radix et Rhizoma rhei* anthraquinones could significantly reduce blood levels of total cholesterol, triglycerides, HDL and LDL.

Conclusion: The optimum extraction process was two times extraction of *Radix et Rhizoma rhei* with 70% ethanol, the amounts of solvent of 8 folds and 5 folds, successively, and the extraction time of 60 min each. In addition, this extract has an antilipemic effect in mice.

Keywords: Radix et Rhizoma Rhei; total anthraquinones; orthogonal test; anti-lipids

Introduction

Radix et Rhizoma rhei is the dried root and rhizome of *Rheum palmatum* L., *R. tanguticum* Maxim. ex Balf. or *R. officinale* Baill. of the family Polygonaceae, whose major active constituent was anthraquinone derivatives (Li et al., 2006). Radix et Rhizoma Rhei has a gastric mucosal protective effect, major anti-gastritis and anti-peptic ulcer active constituents were emodin, aloe-emodin, chrysophanol, rhein, etc. (Ding et al., 2007; Wang et al., 1997; Zhang, 2008). In recent years, macroporous resin is widely used in the field of purification and refinement of TCM, which is characterized by large adsorption capacity, simple regeneration and reliable method, macroporous resin is especially suitable for purification and isolation of large acute chemical constituents, while achieving a good isolation effect in the refinement of anthraquinones, flavonoids, saponins, alkaloids and other active constituents of TCM, macroporous resin can also significantly reduce the yield of solid matter, and further reduce the dose (Li, 2007; Chen et al., 2009). This study will focus on the investigation of extraction process of total anthraquinones in Radix et Rhizoma Rhei and their antilipemic effects.

Materials and Methods

Instruments and reagents

751G UV spectrophotometer (Shanghai Analytical Instrument Factory); electronic analytical balance (Shanghai Analytical Instrument Factory). All reagents were of analytical grade; and water was distilled water.

Drugs

Radix et Rhizoma Rhei was purchased from Sichuan TCM Decoction Pieces Co., Ltd., which was identified by Professor Lu Feng from the Department of Pharmacognosy of our institute to be consistent with relevant requirements under subparagraph *Radix et Rhizoma rhei* in the "Chinese Pharmacopoeia" 2010 edition Vol. 1. Reference substance emodin (99.8%) was purchased from the National Institute for the Control of

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Pharmaceutical and Biological Products, batch number: G2365-6395.

Animals

24 healthy adult Kunming mice from the SPF center of Qinghai-Normal University, half male and half female, weighing 20~25 g, were randomly divided into three groups. All experimental procedures were approved by the Animal Research Committee.Ethics

Methods and Results

Plotting of standard curves and establishment of content determination method

Emodin reference solution: 4.9 mg of emodin reference substance was accurately weighed, dissolved in an appropriate amount of methanol, diluted to the mark in a 50 mL volumetric flask, and shaken well, which was used as the reference solution. Plotting of standard curves: 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 ml of emodin reference solutions (0.098 mg/ml) were accurately drawn, placed in 25 ml flasks, and stained by addition of 10 ml of 0.5% magnesium acetate methanol solution, respectively. Then their volumes were diluted to 25 ml with methanol, and shaken well. Absorbance was measured at 510 nm with 0.5% magnesium acetate methanol solution as the blank control. Regression equation was calculated as A=3714C+0.00765 R=0.9990

Preparation of test solution

20 ml of extract was accurately drawn, placed in a 250 ml round bottom flask, added with 50 ml of 2% H_2SO_4 and 70 ml of chloroform, and heated and hydrolyzed in a water bath for 2 h, after cooling, chloroform layer was separated and taken, and the hydrolysate was again extracted three times with chloroform, until the color of chloroform layer turned light. The chloroform solutions were combined, adjusted to neutral pH, chloroform was removed, and the residue was dissolved in methanol, and diluted to the mark in a 25ml volumetric flask, which was used in content determination.

Water extraction method

10 g of Radix et Rhizoma Rhei was accurately weighed, and extracted by conventional reflux extraction, each extract was concentrated, respectively, and diluted to the mark in 50 ml volumetric flasks, after treatment in accordance with the method in the "Preparation of test solution", 1 ml was precisely pipetted into a 50 ml volumetric flask, stained, and content was determined and calculated according to the standard curve plotting method in the "Plotting of standard curves and establishment of content determination method" (Table. 1).

Table 1: Total anthraquinone yield by water extraction of Radix et Rhizoma Rhei

Number of times	Water volume (ml)	Time (min)	Total anthraquinone yield (%)
1	80	60	0.845
2	60	30	0.526
3	40	30	0.265
4	40	30	0.254
5	40	30	0.189

Ethanol extraction method

10 g of Radix et Rhizoma Rhei was accurately weighed, and extracted under reflux with 70% ethanol, treatment and content determination methods were the same as in the "Water extraction method"Tables. 2, 3).

Table 2: Total anthraquinone yield by ethanol extraction of Radix et Rhizoma Rhei

Number of times	Solvent volume (ml)	Time (min)	Total anthraquinone yield (%)
1	50	60	2.025
2	40	30	0.512
3	30	30	0.348
4	30	30	0.169

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Table 3: Results of total anthraquinone yield by different extraction methods of Radix et Rhizoma Rhei

Extraction method	Total anthraquinone yield (%)	Extraction rate (%)
Reference medicinal material	5.282	-
Ethanol extraction method	3.054	57.819
Water extraction method	2.079	39.360

Optimization of ethanol extraction process conditions

Investigated factors and their levels

Four factors, namely ethanol concentration, extraction time, ethanol amount and extraction times, were selected, and each factor had three levels; the level of factor table was drawn as shown in Table 4.

Table 4: Levels and factors of ethanol extraction process of total anthraquinones in Radix et Rhizoma Rhei

Level	Factor			
	A Ethanol concentration (%)	B Ethanol amount (folds)	C Extraction time (min)	D Extraction times (times)
1	50%	4, 3, 3	60, 60, 45	1
2	70%	6, 4, 3	60, 45, 30	2
3	90%	8, 5, 4	45, 30, 30	3

Note: if D is 1 time, it corresponds to the first digit values of B, C; if D is 2 times, it corresponds to the second digit values of B, C; and if D is 3 times, it corresponds to all values of B, C.

Optimization experiment of ethanol extraction process conditions and results

10 g of Radix et Rhizoma Rhei medicinal material was separately weighed, extracted, concentrated and then diluted to the mark in 50 ml volumetric flasks according to the $L_9(3^4)$ orthogonal design program, test solution was prepared under the method prescribed in "Preparation of test solution", and total anthraquinone content was determined according to the method in "Ethanol extraction method". Experimental program and the results are shown in Table 5.

Range analysis results in Tab. 5 show that the factors A, B, C and D had different degrees of influence on extraction of total anthraquinones in Radix et Rhizoma Rhei, which were A>C>B>D, the combination of optimum extraction process was: three times of extraction with 70% ethanol, solvent amount of 8, 5 and 4 folds and extraction time of 45, 30 and 30 min, respectively. ANOVA was performed with total anthraquinone yield as an index. The results are shown in Table 6.

ANOVA results showed that factor A had a significant influence on the extraction of total anthraquinones in Radix et Rhizoma Rhei, after comprehensive consideration of various factors and actual production, the optimum extraction process of total anthraquinones in Radix et Rhizoma Rhei was determined as $A_2B_2C_1D_2$, i.e. two times extraction with 70% ethanol, a 8-fold amount of solvent in the first extraction, and a 5-fold amount of solvent in the second extraction, as well as extraction time of 60 min each. Scale-up experiment on the determined process conditions showed that the yield was basically stable.

Antilipemic effect of total anthraquinones in Radix et Rhizoma Rhei (Luo et al., 2006; Jin et al., 2006; Dong, 2006)

Administration method

Mice were administered i.g. with 3.0 g/kg, 5.0 g/kg and 10.0 g/kg of total Radix et Rhizoma Rhei anthraquinone extracts or an equivalent volume of saline daily for a total of 10 d, 1 h after the last administration, blood samples were collected to determine blood levels of total cholesterol, triglycerides, HDL and LDL.

Determination method

Total cholesterol and triglyceride levels were determined by enzymatic colorimetry; HDL and LDL levels were determined by clearance

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method. The results are shown in Tables 7 and 8.

Table 5: Orthogonal experimental program and results

Test No.	A	B	C	D	Total anthraquinone yield (%)
1	1	1	1	1	0.875
2	1	2	2	2	0.876
3	1	3	3	3	0.901
4	2	1	2	3	1.402
5	2	2	3	1	1.180
6	2	3	1	2	1.670
7	3	1	3	2	1.063
8	3	2	1	3	1.312
9	3	3	2	1	1.315
I	2.632	3.339	3.879	3.356	
II	4.230	3.354	3.569	3.589	
III	3.679	3.912	3.126	3.721	
R	1.632	0.543	0.756	0.256	

Table 6: ANOVA table

Source of variation	Sum of Squares	Degrees of Freedom	Variance	F value	Significance
A	0.456	2	0.230	38.18	
B	0.065	2	0.035	5.66	
C	0.090	2	0.044	7.49	
Error	0.012	2	0.006		

$F_{0.05}(2, 2)=19.00$, $F_{0.01}(2, 2)=99.00$

Table 7: Effects of total Radix et Rhizoma Rhei anthraquinones on serum levels of total cholesterol and triglycerides

Group	Total cholesterol	Triglycerides
Normal saline	2.831±0.18	1.468±0. 10
Small dose	2.401±0. 16*	1.203±0. 13*
Medium dose	2.212±0. 15**	1.152±0. 08**
Large dose	2.020±0. 14**	1.023±0. 09**

Comparison with the normal saline group, *P<0.05; **P<0.01; n=8

As can be seen from Tab. 7, total Radix et Rhizoma Rhei anthraquinones could significantly reduce serum total cholesterol and triglyceride levels, and their effects exhibited apparent dose-dependence. As can be seen from Tab. 8, total Radix et Rhizoma Rhei anthraquinones could significantly reduce serum HDL and LDL levels, and their effects exhibited apparent dose-dependence.

Table 8: Effects of total Radix et Rhizoma Rhei anthraquinones on serum levels of HDL and LDL (x±s) mmol/L

Group	HDL	LDL
Normal saline	1.02±0.08	0.46±0. 02
Small dose	1.18±0. 10**	0.35±0. 03*
Medium dose	1.29±0. 11**	0.30±0. 02**
Large dose	1.50±0. 11**	0.31±0. 03**

Comparison with the normal saline group, *P<0.05; **P<0.01; n=8

Discussion

Radix et Rhizoma Rhei was extracted with water and ethanol, respectively, and total anthraquinone content was measured, which was higher in the latter, indicating that ethanol was a more ideal extracting solvent. Content determination was performed according to the method

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prescribed in the Chinese Pharmacopoeia, and total anthraquinone content in medicinal Radix et Rhizoma Rhei was determined to be 5.282%. After comparison between water and ethanol extraction processes, ethanol extraction process was determined to be more desirable. Analysis of orthogonal experimental results showed that the ethanol concentration had a significant influence on extraction of total Radix et Rhizoma Rhei anthraquinones, after comprehensive consideration of various factors, and combined with actual production, the optimum extraction process of total anthraquinones in Radix et Rhizoma Rhei was determined as two times extraction with 70% ethanol, solvent amount of 8 and 5 folds, successively, and extraction time of 60 min each.

The present study found that total Radix et Rhizoma Rhei anthraquinones could reduce serum total cholesterol level, increase HDL level, lower LDL and VLDL levels, reduce LDL and prevent excessive LDL oxidation in mice. Total Radix et Rhizoma Rhei anthraquinones reduce fat deposition in the coronary artery wall through the above actions, thereby reducing the incidence of atherosclerosis. The results showed that total reducing the incidence of anthraquinones can markedly reduce blood lipid level, lower serum total cholesterol level, increase HDL level and lower LDL and VLDL levels in mice. Total reducing the incidence of anthraquinones have a significant hypolipidemic effect.

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